DETECTION OF SPONTANEOUSLY OCCURING AMYLOID PLAQUES IN A PRIMATE MODEL OF ALZHEIMER'S DISEASE

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INTRODUCTION:

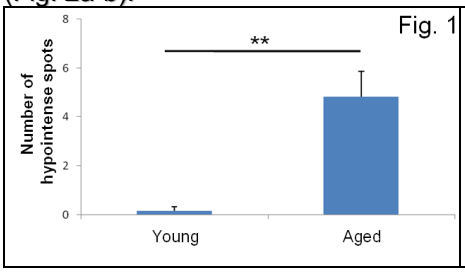
Alzheimer's disease (AD) is the most common type of neurodegenerative diseases. Amyloid deposits/plaques, one of its characteristic lesions, constitute the main target for diagnostics and therapeutics and have previously been identified in transgenic mouse models of AD using magnetic resonance (MR) microscopy [1,2]. These plaques typically appear as hypointense spots on T2 or T2*-weighted MR images and their sizes range from 50 µm to 200 µm. Plaques from humans are however very different than those of rodents. For example they are less compact and their detection is expected to be much more difficult than those of rodents. As a first step toward imaging amyloid plaques in humans, we evaluated plaques detection in a Primate model (the mouse lemur (Microcebus murinus)) developing spontaneously amyloid plaques [3]. Adult animals weight approximately 100 g and have a brain that measures approximately 23 mm, with a maximum width of 18 mm. These characteristics, added to their phylogenic proximity with humans, make them a promising model to evaluate protocols to detect amyloid plaques in humans. To detect amyloid plaques in mice, some investigators have used targeted contrast agents that selectively bind to amyloid plaques [4]. Such agents are not readily available and cannot yet be clinically used. Various studies demonstrated that a non-targeted contrast agent such as Gadolinium-DOTA (Gd or passive staining protocols) coupled with a very high resolution allows to identify ex-vivo but also in–vivo amyloid deposits in the brains of the transgenic mice [5,6]. Here we demonstrate that this method allows to detect spontaneously occurring amyloid plaques in the brain of aged primates.

METHODS:

The brain of 12 mouse lemurs aged from 1 to 10 years (n=6 young and 6 aged (mean ages=2.5 and 7.5 years)) were Gd-stained by being soaked in a solution of PBS with Dotarem© (Guerbet, France) at a concentration of 2.5 mmol.L-1, during at least 24 hours. For MRI acquisition, brains were removed from the solution and placed in a container filled with Fluorinert© (3M, US). MR images were acquired on a 7T wholebody MRI system (Siemens, Syngo MR VB15) with maximum gradient strength of 80 mT/m and a slew rate of 333 mT/m/s. A 3D gradient-echo-based sequence was used (FLASH, TR/TE = 200/20 ms, scan time = 5-6 h depending on the brain size, resolution = 31x31x120 µm). Hypointense spots possibly corresponding to amyloid plaques were searched on the images. Following the MRI, the brains were evaluated by neuropathology. Immunohistochemistry was performed to detect amyloid plaques (40µm thick sections, 4G8 antibody (SIGNET)). Perls-DAB staining was also performed to detect iron deposits on histological sections.

RESULTS:

Following Gd-staining several hypointense spots could be detected in the cortex and hippocampus of mouse lemurs. The number of spots were higher in aged animals as compared to young ones (Fig. 1; Mann Whitney's test, U=0.5; p<0.005). The number of spots was correlated with the age of the animals. Amyloid deposits could be identified by histology in one animal and registration of MR and histological sections showed that amyloid plaques appeared as hypointense spots on MR images (Fig. 2a-b). Iron staining was low although not null at the level of amyloid plaques that were detected by MRI (Fig. 2a-c). Many hypointense spots could not be registered to amyloid plaques, but could be associated to blood vessels (Fig. 2a-b).



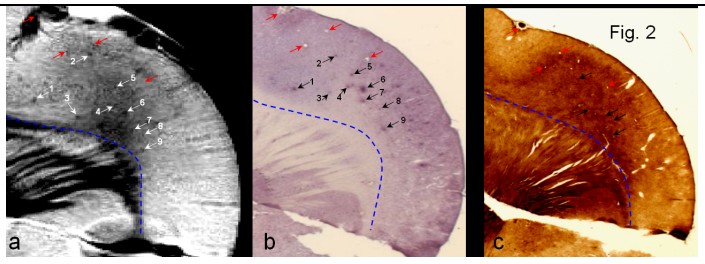


Fig. 1. Number of hypointense spots detected in the cortex and hippocampus of young and old animals.

Fig. 2. Registration between hypointense MR spots (a), amyloid deposits (b-white arrows), blood vessels (b-red arrows), and iron deposits (c, arrows).

CONCLUSION:

Here we show that high resolution MRI and Gd-staining allows detecting spontaneously occurring amyloid plaques in primates. The most probable explanation for this phenomenon is that its hydrophilic properties makes Gd diffuse easily in the grey matter, which is hydrophilic, and less easily in the hydrophobic amyloid plaques. This works thus extends to "normal" amyloid plaques from aged primates results showing the ability of the Gd-staining method to detect plaques in-vivo and ex-vivo in transgenic mouse models of AD. Hypointense spots, not related to amyloid plaques, are also detected during normal aging in aged lemur primates. They might be caused by blood vessels and possibly microhemorrhages.

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